

WHAT IS CLAIMED IS:

- 5           1. A polypeptide conjugate comprising:
- (i) a polypeptide exhibiting G-CSF activity, the polypeptide comprising an amino acid sequence that differs from the amino acid sequence shown in SEQ ID NO:1 in at least one substitution selected from the group consisting of T1K, P2K, L3K, G4K, P5K, A6K, S7K, S8K, L9K, P10K, Q11K, S12K, F13K, L14K, L15K, E19K, Q20K, V21K, Q25K, G26K, D27K, 10 A29K, A30K, E33K, A37K, T38K, Y39K, L41K, H43K, P44K, E45K, E46K, V48K, L49K, L50K, H52K, S53K, L54K, I56K, P57K, P60K, L61K, S62K, S63K, P65K, S66K, Q67K, A68K, L69K, Q70K, L71K, A72K, G73K, S76K, Q77K, L78K, S80K, F83K, Q86K, G87K, Q90K, E93K, G94K, S96K, P97K, E98K, L99K, G100K, P101K, T102K, D104K, T105K, Q107K, L108K, D109K, A111K, D112K, F113K, T115K, T116K, W118K, Q119K, Q120K, 15 M121K, E122K, E123K, L124K, M126K, A127K, P128K, A129K, L130K, Q131K, P132K, T133K, Q134K, G135K, A136K, M137K, P138K, A139K, A141K, S142K, A143K, F144K, Q145K, S155K, H156K, Q158K, S159K, L161K, E162K, V163K, S164K, Y165K, V167K, L168K, H170K, L171K, A172K, Q173K and P174K, and
- (ii) at least one non-polypeptide moiety attached to a lysine residue of the 20 polypeptide.
2. The conjugate of claim 1, wherein at least one of amino acid residues K16, K23, K34 and K40 of SEQ ID NO:1 has been deleted or substituted with another amino acid residue.
3. The conjugate of claim 2, wherein at least one of K16, K23, K34 and K40 25 has been substituted with an R or Q residue.
4. The conjugate of claim 1, wherein the polypeptide comprises at least one substitution selected from the group consisting of P5K, A6K, S7K, S8K, L9K, P44K, E45K, E46K, V48K, L49K, L50K, H52K, S53K, L54K, I56K, P57K, P60K, L61K, S62K, S63K, P65K, S66K, Q67K, A68K, L69K, Q70K, L71K, A72K, G73K, S76K, Q77K, L78K, S80K, 30 F83K, Q86K, G87K, Q90K, E93K, G94K, S96K, P97K, E98K, L99K, P101K, D104K, T105K, Q107K, L108K, D109K, A111K, D112K, F113K, T115K, T116K, W118K, Q119K, Q120K,

5 M121K, E122K, E123K, L124K, M126K, A127K, P128K, A129K, L130K, Q131K, P132K, T133K, Q134K, G135K, A136K, M137K, P138K, A139K, A141K, S142K, A143K, F144K, Q145K, S155K, H156K, Q158K, S159K, L161K, E162K, V163K, S164K, Y165K, V167K, L168K, H170K, L171K, A172K, Q173K and P174K.

10 5. The conjugate of claim 4, wherein the polypeptide comprises at least one substitution selected from the group consisting of Q70K, Q90K, T105K, Q120K and T133K.

6. A conjugate comprising:

(i) a polypeptide exhibiting G-CSF activity, the polypeptide comprising an amino acid sequence that differs from the amino acid sequence of hG-CSF shown in SEQ ID NO:1 in at least one substitution selected from the group consisting of T1C, P2C, L3C, G4C, P5C, A6C, 15 S7C, S8C, L9C, P10C, Q11C, S12C, F13C, L14C, L15C, E19C, Q20C, V21C, R22C, Q25C, G26C, D27C, A29C, A30C, E33C, A37C, T38C, Y39C, L41C, H43C, P44C, E45C, E46C, V48C, L49C, L50C, H52C, S53C, L54C, I56C, P57C, P60C, L61C, S62C, S63C, P65C, S66C, Q67C, A68C, L69C, Q70C, L71C, A72C, G73C, S76C, Q77C, L78C, S80C, F83C, Q86C, G87C, Q90C, E93C, G94C, S96C, P97C, E98C, L99C, G100C, P101C, T102C, D104C, T105C, 20 Q107C, L108C, D109C, A111C, D112C, F113C, T115C, T116C, W118C, Q119C, Q120C, M121C, E122C, E123C, L124C, M126C, A127C, P128C, A129C, L130C, Q131C, P132C, T133C, Q134C, G135C, A136C, M137C, P138C, A139C, A141C, S142C, A143C, F144C, Q145C, R146C, R147C, S155C, H156C, Q158C, S159C, L161C, E162C, V163C, S164C, Y165C, R166C, V167C, L168C, R169C, H170C, L171C, A172C, Q173C and P174C, and 25 (ii) at least one non-polypeptide moiety attached to a cysteine residue of the polypeptide.

7. A conjugate comprising

(i) a polypeptide exhibiting G-CSF activity, the polypeptide comprising an amino acid sequence that differs from the amino acid sequence shown in SEQ ID NO:1 in at least one 30 substitution selected from the group consisting of T1D/E, P2D/E, L3D/E, G4D/E, P5D/E, A6D/E, S7D/E, S8D/E, L9D/E, P10D/E, Q11D/E, S12D/E, F13D/E, L14D/E, L15D/E, K16D/E, Q20D/E, V21D/E, R22D/E, K23D/E, Q25D/E, G26D/E, A29D/E, A30D/E, K34D/E, A37D/E, T38D/E, Y39D/E, K40D/E, L41D/E, H43D/E, P44D/E, V48D/E, L49D/E, L50D/E, H52D/E,

5 S53D/E, L54D/E, I56D/E, P57D/E, P60D/E, L61D/E, S62D/E, S63D/E, P65D/E, S66D/E,  
Q67D/E, A68D/E, L69D/E, Q70D/E, L71D/E, A72D/E, G73D/E, S76D/E, Q77D/E, L78D/E,  
S80D/E, F83D/E, Q86D/E, G87D/E, Q90D/E, G94D/E, S96D/E, P97D/E, L99D/E, G100D/E,  
P101D/E, T102D/E, T105D/E, Q107D/E, L108D/E, A111D/E, F113D/E, T115D/E, T116D/E,  
W118D/E, Q119D/E, Q120D/E, M121D/E, L124D/E, M126D/E, A127D/E, P128D/E,  
10 A129D/E, L130D/E, Q131D/E, P132D/E, T133D/E, Q134D/E, G135D/E, A136D/E, M137D/E,  
P138D/E, A139D/E, A141D/E, S142D/E, A143D/E, F144D/E, Q145D/E, R146D/E, R147D/E,  
S155D/E, H156D/E, Q158D/E, S159D/E, L161D/E, V163D/E, S164D/E, Y165D/E, R166D/E,  
V167D/E, L168D/E, R169D/E, H170D/E, L171D/E, A172D/E, Q173D/E and P174D/E, and  
(ii) at least one non-polypeptide moiety attached to an aspartic acid or glutamic  
15 acid residue of the polypeptide.

8. The conjugate of claim 7, wherein the polypeptide comprises at least one  
substitution selected from the group consisting of Q67D/E, Q70D/E, Q77D/E, Q86D/E, Q90D/E,  
Q120D/E, Q131D/E, Q134D/E, Q145D/E and Q173D/E; and/or at least one substitution selected  
from the group consisting of D27N, D104N, D109N, D112N, E19Q, E33Q, E45Q, E46Q, E93Q,  
20 E98Q, E122Q, E123Q and E163Q.

9. The conjugate of claim 1, wherein the non-polypeptide moiety is selected  
from the group consisting of natural and synthetic homopolymers and heteropolymers.

10. The conjugate of claim 9, wherein the polymer is a synthetic homopolymer  
or heteropolymer selected from the group consisting of linear or branched polyethylene glycol,  
25 polyvinylalcohol (PVA), poly-carboxylic acids and poly-(vinylpyrrolidone).

11. The conjugate of claim 10, wherein the polymer is a linear or branched  
polyethylene glycol.

12. The conjugate of claim 11, wherein the polyethylene glycol has a molecular  
weight of about 1000-15,000 Da.

30 13. The conjugate of claim 11, comprising 2-8 polyethylene glycol moieties.

5           14. The conjugate of claim 1, wherein the polypeptide differs in 1-15 amino acid residues from the amino acid sequence shown in SEQ ID NO:1.

          15. The conjugate of claim 14, wherein the polypeptide differs in 2-10 amino acid residues from the amino acid sequence shown in SEQ ID NO:1.

          16. The conjugate of claim 1, wherein the polypeptide is glycosylated.

10           17. The conjugate of claim 16, wherein the polypeptide comprises at least one non-naturally occurring glycosylation site.

          18. A conjugate comprising a glycosylated polypeptide exhibiting G-CSF activity, the polypeptide comprising an amino acid sequence that differs from the amino acid sequence shown in SEQ ID NO:1 in that at least one non-naturally occurring glycosylation site  
15 has been introduced into the amino acid sequence by way of at least one substitution selected from the group consisting of L3N+P5S/T, P5N, A6N, S8N+P10S/T, P10N, Q11N+F13S/T, S12N+L14S/T, F13N+L15S/T, L14N+K16S/T, K16N+L18S/T, E19N+V21S/T, Q20N+R22S/T, V21N+K23S/T, R22N+I24S/T, K23N+Q25S/T, Q25N+D27S/T, G26N+G28S/T, D27N+A29S/T, A29N+L31S/T, A30N+Q32S/T, E33N+L35S/T, A37N+Y39S/T,  
20 T38N+K40S/T, Y39N+L41S/T, P44N+E46S/T, E45N+L47S/T, E46N+V48S/T, V48N+L50S/T, L49N+G51S/T, L50N+H52S/T, H52N+L54S/T, S53N+G55S/T, P60N, L61N, S63N+P65S/T, P65N+Q67S/T, S66N+A68S/T, Q67N+L69S/T, A68N+Q70S/T, L69N+ L71S/T, Q70N+A72S/T, L71N+G73S/T, G73N+L75S/T, S76N+L78S/T, Q77N+H79S/T, L78N, S80N+L82S/T, F83N+Y85S/T, Q86N+L88S/T, G87N+L89S/T, Q90N+L92S/T, E93N+I95S/T,  
25 P97N+L99S/T, L99N+P101S/T, P101N+L103S/T, T102N+D104S/T, D104N+L106S/T, T105N+Q107S/T, Q107N+D109S/T, L108N+V110S/T, D109N+A111S/T, A111N+F113S/T, D112N+A114S/T, F113N, T115N+I117S/T, T116N+W118S/T, W118N+Q120S/T, Q119N+M121S/T, Q120N+E122S/T, M121N+E123S/T, E122N+L124S/T, E123N+G125S/T, L124N+M126S/T, M126N+P128S/T, P128N+L130S/T, L130N+P132S/T, P132N+Q134S/T,  
30 T133N+G135S/T, Q134N+A136S/T, A136N+P138S/T, P138N+F140S/T, A139N+A141S/T, A141N+A143S/T, S142N+F144S/T, A143N+Q145S/T, F144N+R146S/T, Q145N+R147S/T, R146N+A148S/T, R147N+G149S/T, S155N+L157S/T, H156N+Q158S/T, S159N+L161S/T, L161N+V163S/T, E162N, V163N+Y165S/T, S164N+R166S/T, Y165N+V167S/T,

5 R166N+L168S/T, V167N+R169S/T, L168N+H170S/T, R169N+L171S/T and  
H170N+A172S/T.

19. The conjugate of claim 18, comprising at least one substitution selected from  
the group consisting of P5N, A6N, P10N, P60N, L61N, L78N, F113N and E162N; or from the  
group consisting of D27N+A29S, D27N+A29T, D104N+L106S, D104N+L106T,  
10 D109N+A111S, D109N+A111T, D112N+A114S and D112N+A114T.

20. The conjugate of claim 18, wherein two or more glycosylation sites have  
been introduced.

21. The conjugate of claim 18, which further comprises at least one non-  
polypeptide moiety linked to an amino acid residue of the polypeptide, wherein said non-  
15 polypeptide moiety is different from an N- or O-linked carbohydrate moiety.

22. The conjugate of claim 1, which has an increased functional *in vivo* half-life  
and/or serum half-life as compared to rhG-CSF comprising a single N-terminally attached 20  
kDa PEG moiety.

23. The conjugate of claim 1 which fulfils at least one of the following criteria  
20 (A)-(D):

(A) after one subcutaneous administration of 100 microgram per kg body weight  
to rats (based on the weight of the polypeptide part of the conjugate) it:

i) increases formation of white blood cells with at least about the same rate and to  
at least about the same level (measured as number of cells per liter of blood) as administration of  
25 100 microgram of non-conjugated hG-CSF per kg body weight for a period of 12 hours after  
administration, and

ii) increases the level of white blood cells (measured as number of cells per liter  
blood) above the level of white blood cells prior to administration for a period of at least about  
96 hours;

30 (B) after one subcutaneous administration of 25 microgram per kg body weight to  
rats (based on the weight of the polypeptide part of the conjugate) it:

5 i) increases formation of white blood cells with at least about the same rate and to at least about the same level (measured as number of cells per liter of blood) as administration of 100 microgram of non-conjugated hG-CSF per kg body weight for a period of 12 hours after administration, and

10 ii) increases the level of white blood cells (measured as number of cells per liter blood) above the level of white blood cells prior to administration for a period of at least about 72 hours;

(C) after one subcutaneous administration of 100 microgram per kg body weight to rats (based on the weight of the polypeptide part of the conjugate) it:

15 i) increases formation of neutrophils with at least about the same rate and to at least about the same level (measured as number of cells per liter of blood) as administration of 100 microgram of non-conjugated hG-CSF per kg body weight for a period of 12 hours after administration, and

20 ii) increases the level of neutrophils (measured as number of cells per liter blood) above the level of neutrophils prior to administration for a period of at least about 96 hours;

(D) after one subcutaneous administration of 25 microgram per kg body weight to rats (based on the weight of the polypeptide part of the conjugate) it:

25 i) increases formation of neutrophils with at least about the same rate and to at least about the same level (measured as number of cells per liter of blood) as administration of 100 microgram of non-conjugated hG-CSF per kg body weight for a period of 12 hours after administration, and

ii) increases the level of neutrophils (measured as number of cells per liter blood) above the level of neutrophils prior to administration for a period of at least about 72 hours.

24. A polypeptide conjugate exhibiting G-CSF activity, comprising a polypeptide with an amino acid sequence that differs in at least one amino acid residue from the amino acid sequence shown in SEQ ID NO:1 and having at least one non-polypeptide moiety attached to an attachment group of the polypeptide, wherein the polypeptide conjugate has an increased functional *in vivo* half-life and/or serum half-life as compared to rhG-CSF comprising a single N-terminally attached 20 kDa PEG moiety.

5           25. The polypeptide conjugate of claim 24, comprising two or more attached non-polypeptide moieties.

26. A polypeptide conjugate exhibiting G-CSF activity, comprising a polypeptide with an amino acid sequence that differs in at least one amino acid residue from the amino acid sequence shown in SEQ ID NO:1 and having at least one non-polypeptide moiety  
10 attached to an attachment group of the polypeptide, the polypeptide conjugate being characterized in that it fulfils at least one of the following criteria (A)-(D):

(A) after one subcutaneous administration of 100 microgram per kg body weight to rats (based on the weight of the polypeptide part of the conjugate) it:

i) increases formation of white blood cells with at least about the same rate and to  
15 at least about the same level (measured as number of cells per liter of blood) as administration of 100 microgram of non-conjugated hG-CSF per kg body weight for a period of 12 hours after administration, and

ii) increases the level of white blood cells (measured as number of cells per liter blood) above the level of white blood cells prior to administration for a period of at least about  
20 96 hours;

(B) after one subcutaneous administration of 25 microgram per kg body weight to rats (based on the weight of the polypeptide part of the conjugate) it:

i) increases formation of white blood cells with at least about the same rate and to at least about the same level (measured as number of cells per liter of blood) as administration of  
25 100 microgram of non-conjugated hG-CSF per kg body weight for a period of 12 hours after administration, and

ii) increases the level of white blood cells (measured as number of cells per liter blood) above the level of white blood cells prior to administration for a period of at least about  
72 hours;

(C) after one subcutaneous administration of 100 microgram per kg body weight to rats (based on the weight of the polypeptide part of the conjugate) it:

i) increases formation of neutrophils with at least about the same rate and to at least about the same level (measured as number of cells per liter of blood) as administration of  
35 100 microgram of non-conjugated hG-CSF per kg body weight for a period of 12 hours after administration, and

- 5                    ii) increases the level of neutrophils (measured as number of cells per liter blood)  
above the level of neutrophils prior to administration for a period of at least about 96 hours;
- (D) after one subcutaneous administration of 25 microgram per kg body weight to  
rats (based on the weight of the polypeptide part of the conjugate) it:
- 10                   i) increases formation of neutrophils with at least about the same rate and to at  
least about the same level (measured as number of cells per liter of blood) as administration of  
100 microgram of non-conjugated hG-CSF per kg body weight for a period of 12 hours after  
administration, and
- ii) increases the level of neutrophils (measured as number of cells per liter blood)  
above the level of neutrophils prior to administration for a period of at least about 72 hours.
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- 15                   27. A polypeptide exhibiting G-CSF activity, wherein the polypeptide has an  
amino acid sequence as defined in claim 1.
28. A nucleotide sequence encoding the polypeptide of claim 27.
29. An expression vector harbouring the nucleotide sequence of claim 28.
30. A host cell comprising a nucleotide sequence according to claim 28.
- 20                   31. A method for producing a polypeptide conjugate, comprising culturing a  
host cell according to claim 30 under conditions conducive for expression of a polypeptide  
exhibiting G-CSF activity, and recovering the polypeptide, wherein a) the polypeptide comprises  
at least one N- or O-glycosylation site and the host cell is a eukaryotic host cell capable of *in*  
*vivo* glycosylation, and/or b) the polypeptide is subjected to conjugation to a non-polypeptide
- 25                   moiety *in vitro*.
32. A composition comprising a conjugate according to claim 1 and a  
pharmaceutically acceptable carrier or excipient.
33. A method for treating a mammal having a general haematopoietic disorder,  
including disorders arising from radiation therapy or from chemotherapy, treatment of
- 30                   leukopenia, AIDS or other immunodeficiency diseases, and treatment of bacterial or other

5 infections, comprising administering to a mammal in need thereof an effective amount of a conjugate according to claim 1.